

6

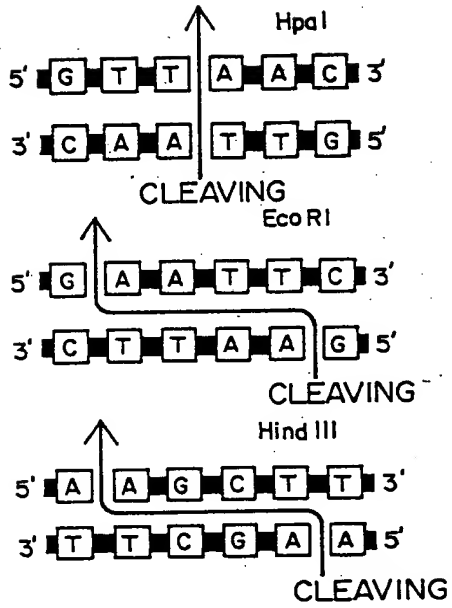


FIG. 1

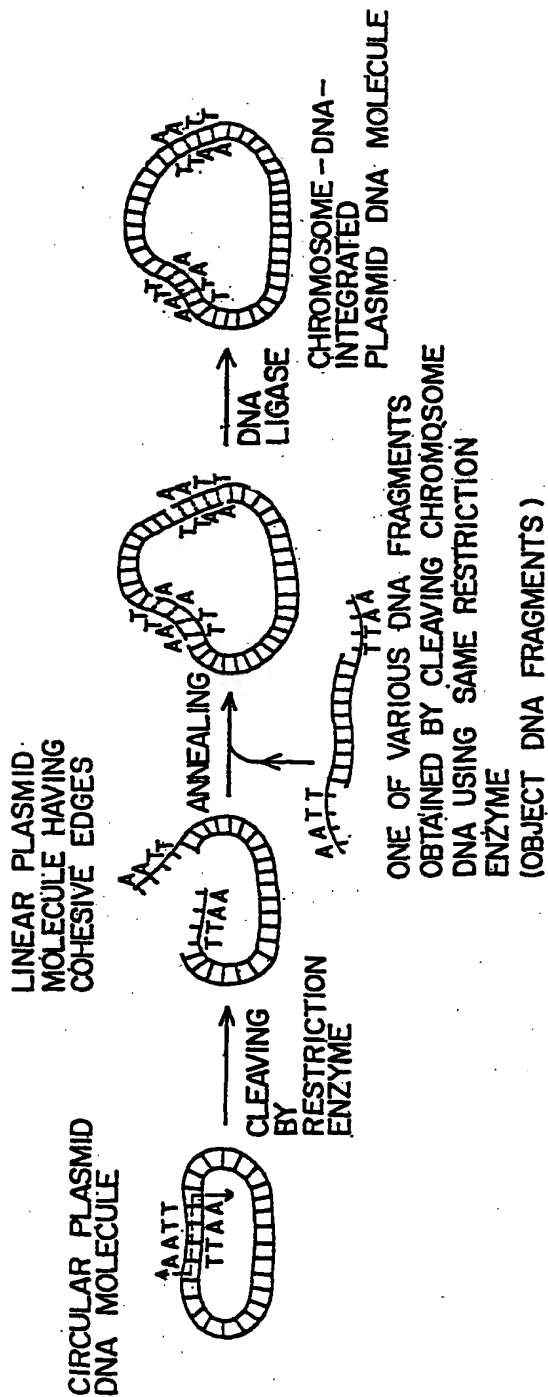


FIG. 2

09765269-052407
104250-69258760

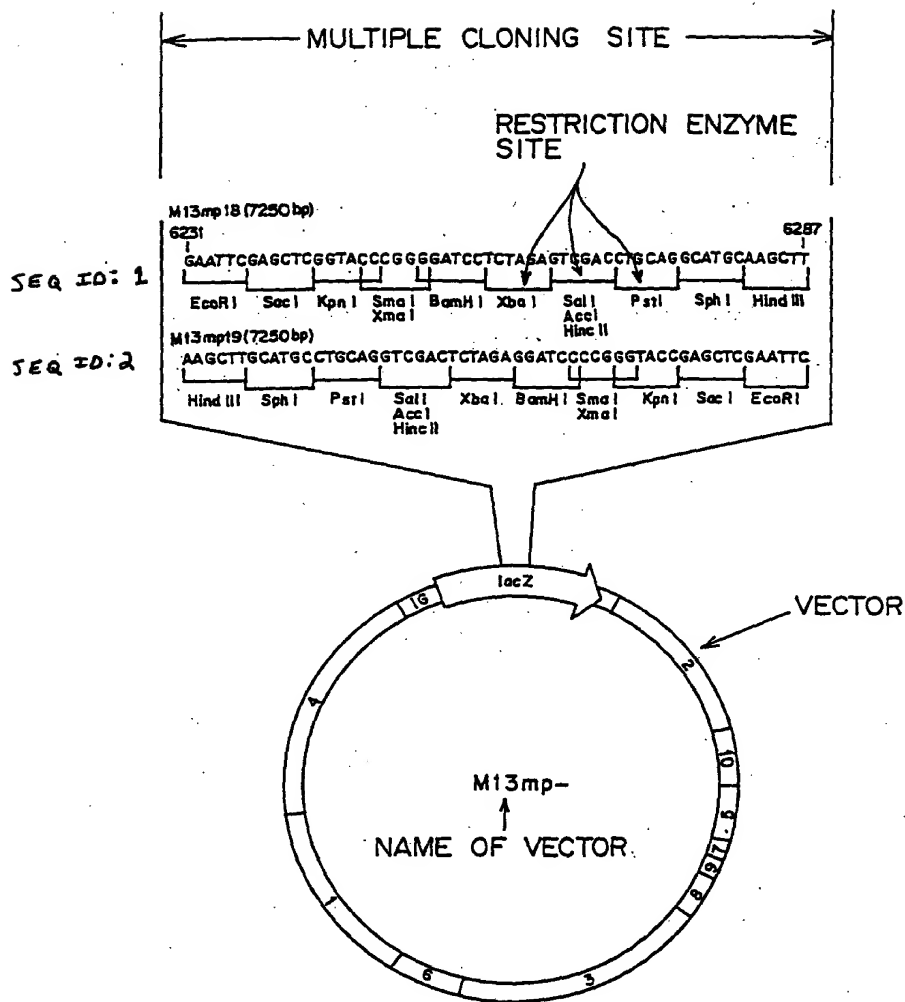
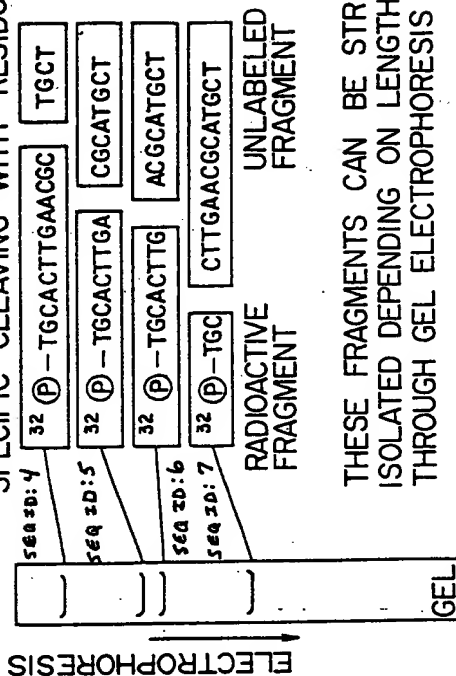


FIG. 3

DNA FRAGMENT LABELED WITH ^{32}P AT 5' EDGE

SEQ ID: 3 ^{32}P - TGCAC TTGAACGCATGCT

RADIOACTIVE FRAGMENTS OF VARIOUS LENGTHS THROUGH CHEMICAL PROCESS OF SPECIFIC CLEAVING WITH RESIDUAL BASE A



THESE FRAGMENTS CAN BE STRICTLY ISOLATED DEPENDING ON LENGTH THROUGH GEL ELECTROPHORESIS

FIG. 4

09785269-052401

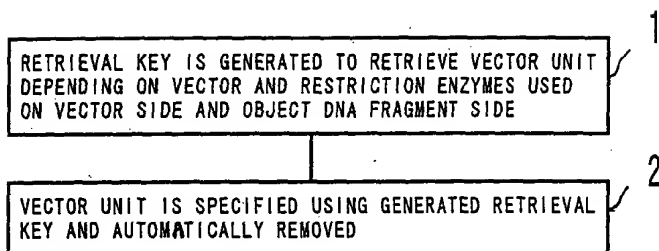


FIG. 5

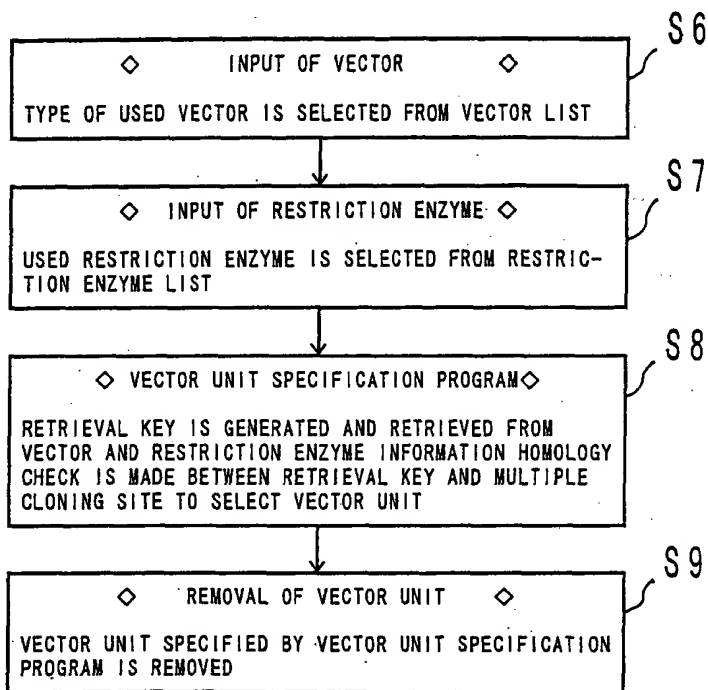


FIG. 6

09785269-052401

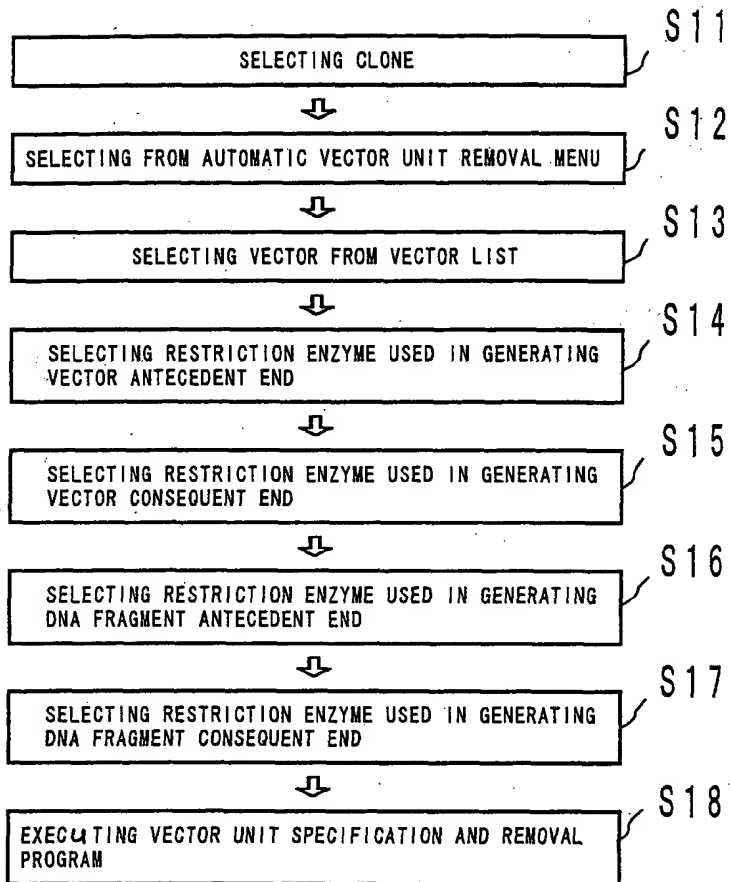


FIG. 7

09785269-052401

M13MP18
M13MP19
PBR322
PSL1180
PSL1190
PT7T318U
PT7T319U
PTZ18R
PTZ19R
PUC18
PUC19, ETC.

FIG. 8

VECTOR DB FORMAT

```
>ID
PUC18
>SEQ ID: 8
TCCGCGGTTTCGGTGATGACGGTGAAAACTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGCTGTGAAGCGGAT
GCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTGGCGGGTGTGGGGTGGCTTAATATGCGGCATCAGA
GCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAAATACCGCATCAGGCGCC
ATTCGCCATTTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGG
GGATGTGCTGCAAGGCGATTAAAGTTGGGTAAACGCCAGGGTTTTCCAGTCACGACGTTGTAAAAACGACGGCCAGTGCCAA
GCTTGATGCTGCGAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCGTAATCATGGTCATAGCTGTTTCCT
GTGTGAAATGTTATCCGCTCACAATCCACACAACATACGAGCGGGAAGCATAAAGTGTAAAGCCTGGGGTGCCTAATG
AGTGAGCTAACTCACATTAAATGCGTTCGCTCACTGCCGCTTTCCAGTCGGGAAACCTGTGTGCCAGCTGCATTAAAT
GAATCGGCCAAACCGCGGGGAGAGGCGGTTTTGCGTATTGGGCGCTCTTCGCTTCCTCGCTCACTGACTCGCTGCGCTCG
GTCGTTGCGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAAATACGGTTATCCACAGAAATCAGGGGATAACGACGG
AAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCC
GCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCG
TTTTCCCTTGGAAGCTCCCTCGTGGCTCTCCTGTTCCGACCTGCCGCTTACCGGATACCTGTCCGCTTTCTCCCTTC
GGGAAGCGTGGCGTTTTCTCAAAGCTCAGCGTGTAGGTATCTCAAGTTCGGTGAAGTTCGTTCCGCTCAAAGCTGGGCTGTG
TGCAGGAACCCCCGTTACGCGCGACCGCTGCGCTTATCCGGTAACTATCGTCTTGAGTCCAAACCCGTAAGACACGAC
TTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTCTTGAAGTG
GTGGCCTAACTACGGCTACACTAGAAGAACAGTATTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAG
TTGGTAGCTCTTGATCCGGCAAAACAAACCACCGCTGGTAGCGGTGGTTTTTTGTTTGAAGCAGCAGATTACGGCAGCA
AAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAACTCACGTTAAGGGAT
TTTGGTCATGAGATTATCAAAAAGGATCTTCACTAGATCCTTTTAAATTAATAAGTTTTAAATCAATCTAAAGTA
TATATGAGTAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTGTTCA
TCCATAGTTGCTGACTCCCCGTGCTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGAT
ACCGCGAGACCCACGCTCACC GGCTCCAGATTATCAGCAATAAACGACGACGCGGAAGGGCCGAGCGCAGAAAGTGGTC
CTGCACTTTATCCGCTCCATCCAGTCTATTAATGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTG
CGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTACGCTCGTGGTTGGTATGGCTTCATTGAGCTCCGGTTCCCA
ACGATCAAGGGCAGTTACATGATCCCCATGTTGTGCAAAAAAGCGGTAGCTCCTTCGGTCTCCGATCGTTGTGAGAA
GTAAGTTGGCCGAGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCTGCCATCCGTAAGATGC
TTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCTC
AATACGGGATAAATACGGCGCCACATAGCAGAACTTTAAAGTGCTCATCTTGGAAAAAGTTCCTCGGGGCGAAAACTCT
CAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAAACCACTCGTGACCCCACTGATCTTCAGCATCTTTTACTTTC
ACCAGCGTTTCTGGGTGAGCAAAAAACAGGAAGGCAAAATGCCGAAAAAGGGAATAAGGGCGACACGGAATGTTGAAT
ACTCACTCTTCTCTTTTCAATATTATTGAAGCAATTATCAGGGTTATTGTCTCATGAGCGGATACATATTGAATGTA
TTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCGAAAAAGTGCCACCTGACGCTCTAAGAAACCATTTATTAC
ATGACATTAACTATAAAAAATAGGCGTATCAGGAGGCCCTTTCGTC
>MULTI
399.. 450
```

FIG. 9

09765269.052401

(* INDICATES MULTIPLE CLONING SITE)

SEQ ID: 9 GTGCCAAGCTTGCATGCCCTGCAGGTCGACTCTAGAGGATCCCCGGGTACCGAGCTCGAATTCGTAAT

SEQ ID: 10 AAGCTT⇒HIND III

SEQ ID: 11 GCATGC⇒SPH I

SEQ ID: 12 CTGCAG⇒PST I

SEQ ID: 13 GTCGAC⇒SAL I, ACC I, HINC II

SEQ ID: 14 TCTAGA⇒XBA I

SEQ ID: 15 GGATCC⇒BAMH I

SEQ ID: 16 CCCGGG ⇒SMA I, XMA I

SEQ ID: 17 GGTACC ⇒KPN I

SEQ ID: 18 GAGCTC ⇒SAC I

SEQ ID: 19 GAATTC ⇒BCOR I

FIG. 10

09785269-052401

VECTOR SIDE

HIND III

SPH I

PST I

SAL I

ACC I

HINC II

XBA I

BAMH I

SMA I

XMA I

KPN I

SAC I

ECOR I

OBJECT DNA
FRAGMENT SIDE

HINDIII

SPH I

PST I

SAL I

ACC I

HINC II

XBA I

BAMH I

SMA I

XMA I

KPN I

SAC I

ECOR I

OTHER RESTRICTION
ENZYME

...

FIG. 11

09785269-052401
104250-69258260

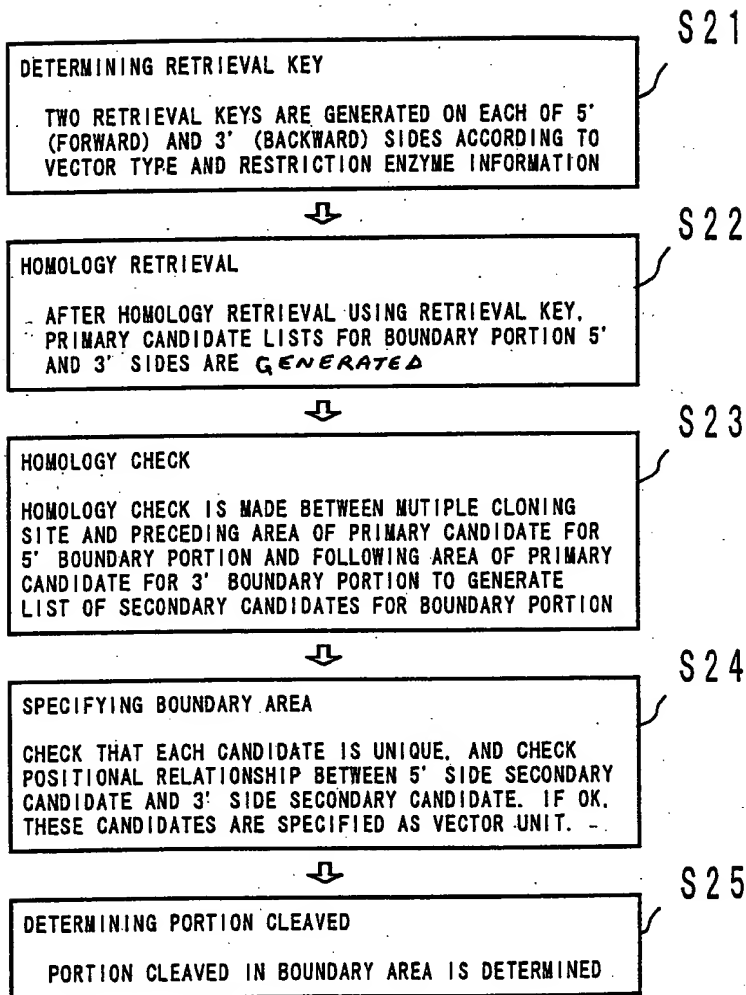


FIG. 12

WHEN SINGLE-STRANDED AREA IS FOUND ON 3' SIDE

STRAND A 5'	AREA A		AREA B3	AREA C	3'
	AREA C		AREA B3	AREA A	5'
STRAND B 3'					
← RESTRICTION ENZYME →					
SITE					

FIG. 13A

WHEN NO SINGLE-STRANDED AREA IS FOUND

STRAND A 5'	AREA A		AREA C	3'
	AREA C			
STRAND B 3'	AREA C		AREA A	5'
	AREA A			
← RESTRICTION ENZYME →				
SITE				

FIG. 13B

WHEN SINGLE-STRANDED AREA IS FOUND ON 5' SIDE

STRAND A 5'	AREA A		AREA B5	AREA C	3'
	AREA C				
STRAND B 3'	AREA C		AREA B5	AREA A	5'
	← RESTRICTION ENZYME →				
					SITE

FIG. 13C

104250-69258/60

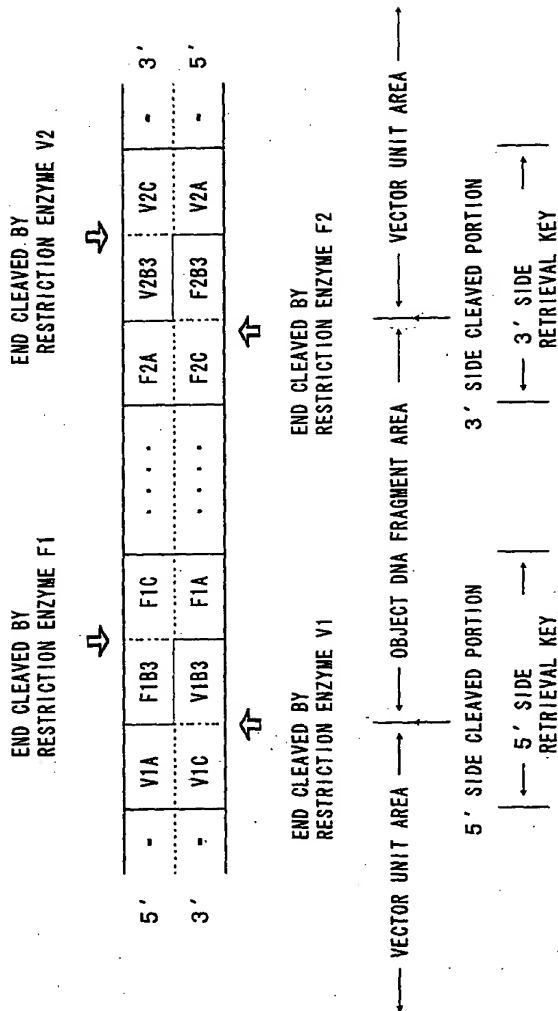


FIG. 14

00765269-052404

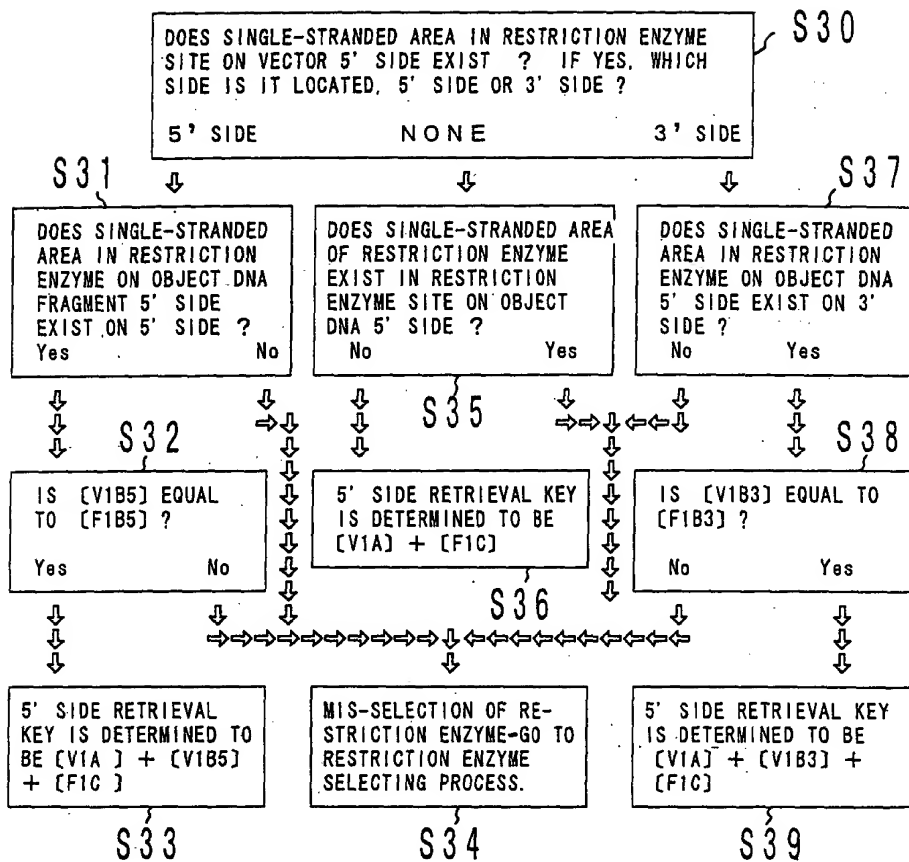


FIG. 15

104250-69258760

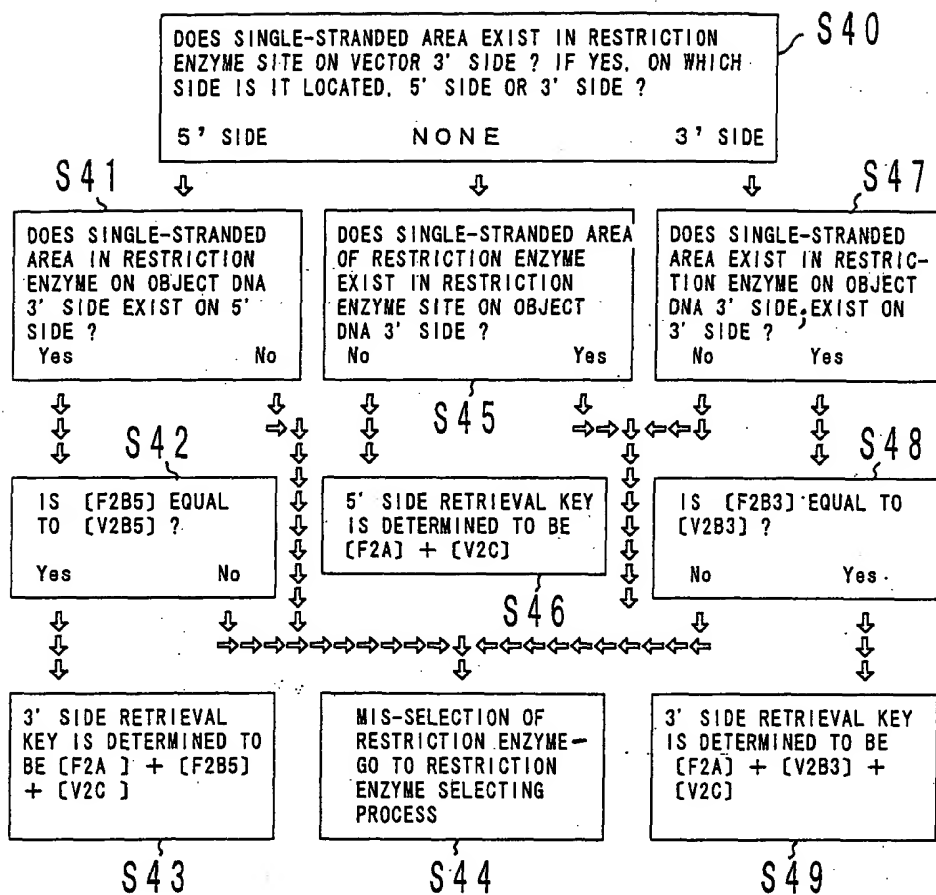


FIG. 16

WHEN HIND III IS SPECIFIED ON VECTOR 5' SIDE
 XBA I IS SPECIFIED ON VECTOR 3' SIDE, HIND III IS
 SPECIFIED ON OBJECT DNA 5' SIDE, AND XBA I IS
 SPECIFIED ON OBJECT DNA 3' SIDE

(**** INDICATES RESIDUAL MULTIPLECLONING SITE
 (++++ INDICATES AN OBJECT DNA FRAGMENT

****	*****
GTGCAAGCTT+++++	TCTAGAGGATCCCCGGTACCGAGCTCGAATTCGTAAT
AAGCTT	TCTAGA
↑	↑

5' SIDE RETRIEVAL KEY
 (IN THIS EXAMPLE,
 HIND III SITE)

9' SIDE RETRIEVAL KEY
 (IN THIS EXAMPLE, XBA I SITE)

FIG. 17

09785269-052401

09785269-052401

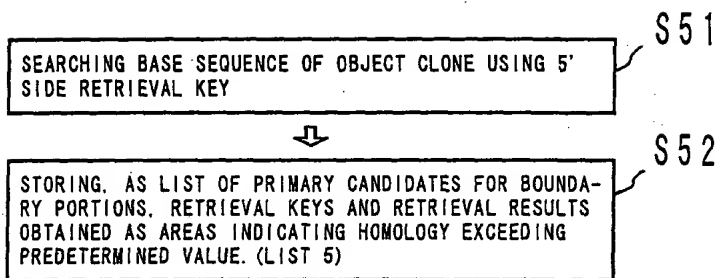


FIG. 18

09765269-052401

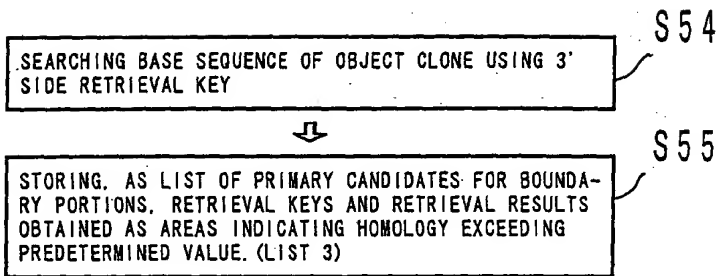


FIG. 19

09705269-052401

DEFINING, IN MULTIPLE CLONING SITE OF VECTOR, RESTRICTION ENZYME SITE USED IN SHEARING 5' SIDE IN MULTIPLE CLONING SITE OF VECTOR AND AREA OUTSIDE ON 5' SIDE AS 5' SIDE RESIDUAL MULTIPLE CLONING SITE (5MCS)

S 6 1



WHEN VECTOR DB CONTAINS BASE SEQUENCE OTHER THAN MULTIPLE CLONING SITE, SUM OF 5MCS AND 5 BASES ON 5' SIDE FROM 5MCS IS DEFINED AS 5' SIDE RESIDUAL VECTOR AREA (5VA). IF VECTOR DB CONTAINS ONLY BASE SEQUENCE OF MULTIPLE CLONING SITE IN VECTOR DB, THEN 5 MCS IS 5VA.

S 6 2

(A HOMOLGY CHECK IS MADE ACCORDING TO FOLLOWING FLOWCHART ON ALL ELEMENTS IN PRIMARY CANDIDATES FOR BOUNDARY PORTIONS (LIST 5) OBTAINED IN 5' SIDE HOMOLGY RETRIEVAL)

DEFINING EACH CANDIDATE IN LIST 5 AND SEQUENCE AREA OUTSIDE ON 5' SIDE AS HOMOLGY CHECK AREA (5HCA) FOR CORRESPONDING CANDIDATE

S 6 3



COMPARING NUMBER OF BASES IN 5' SIDE RESIDUAL VECTOR AREA (5VA), NUMBER OF BASES OF 5HCA, AND NUMBER OF BASES 20, AND DEFINING SMALLEST NUMBER OF BASES AS NUMBER OF BASES FOR USE IN HOMOLGY CHECK (HCB)

S 6 4



EXTRACTING HCB BASES FROM 3' SIDE OF 5VA TO-CHECK HOMOLGY TO HCB BASES ON 3' SIDE OF 5HCA

S 6 5



WHEN CONSTANT HOMOLGY IS OBTAINED, EXTRACTED BASES ARE DEFINED AS SECONDARY CANDIDATES FOR 5' SIDE BOUNDARY PORTIONS.

S 6 6

F I G . 2 0

05401

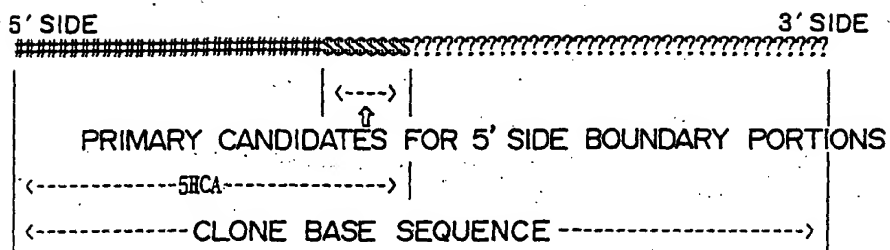


FIG. 21

09765269-052401

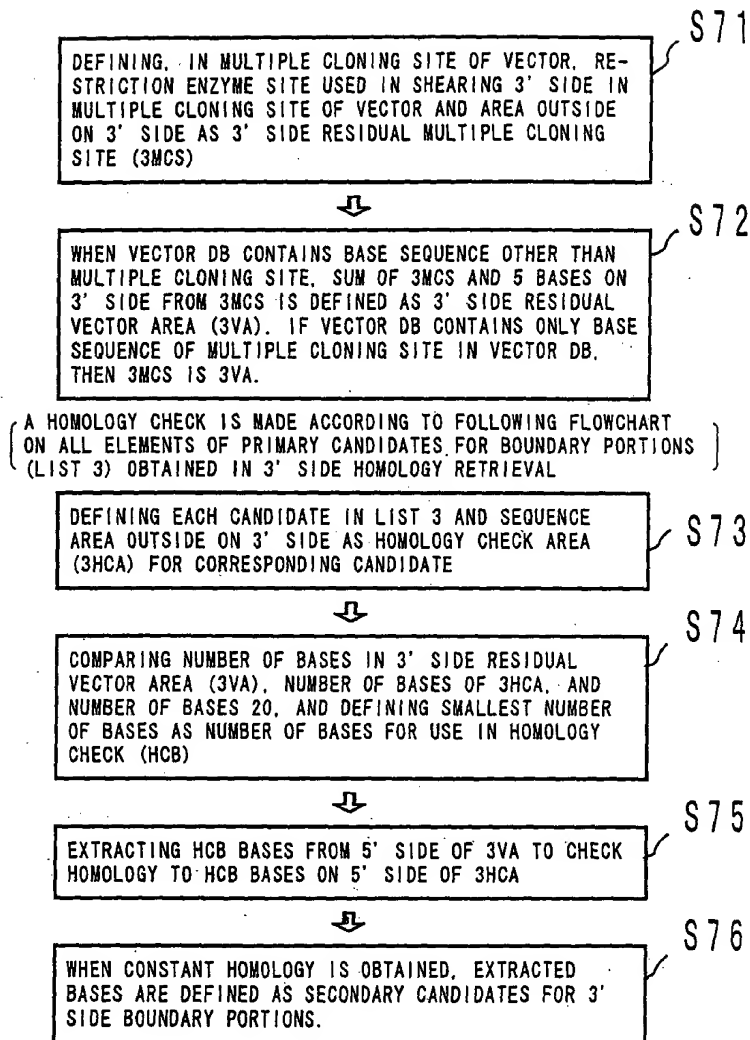


FIG. 22

09785269.052404

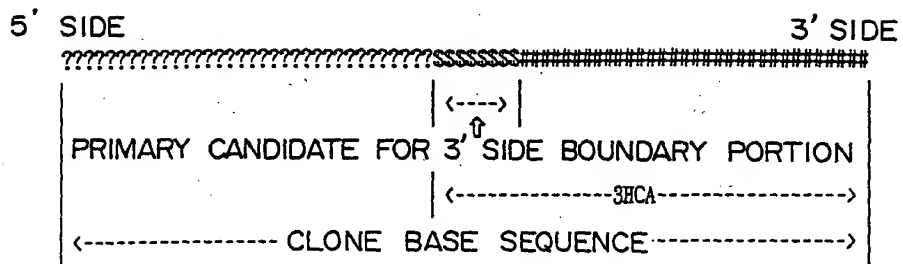


FIG. 23

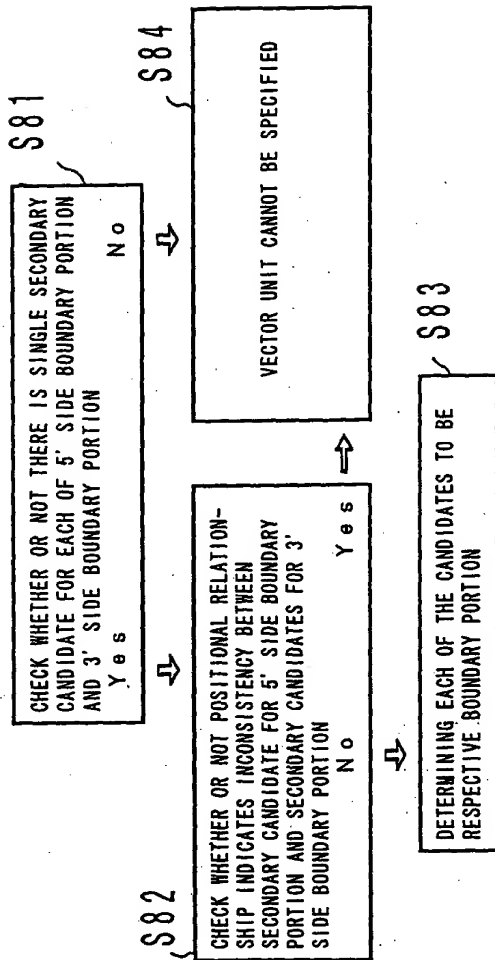


FIG. 24

DISPLAY 30

